Clinical Significance of Low Serum Cryptococcal Antigen Titers by Lateral Flow Assay in Immunocompromised Patients: a Retrospective Case-Control Study

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ABSTRACT *Cryptococcus* species are associated with invasive fungal infections in immunosuppressed individuals. The clinical significance of low-titer cryptococcal antigen (CrAg) by lateral flow assay is frequently uncertain. We investigated the correlation of low CrAg titers with disease in an immunocompromised patient population. Patients with first-time positive CrAg results with low serum titers ($\leq 1:10$) at two medical centers (Los Angeles, CA) from April 2014 to July 2018 were included. Age-matched controls with high ($\geq 1:20$) and negative titers were selected. We extracted medical records for pertinent clinical, radiologic, and laboratory data for cryptococcal disease. From 2,196 serum samples submitted for CrAg testing, 96 cases were included (32 each in low-titer, high-titer, and negative-titer groups). One or more immunocompromising condition was identified in 95% of patients, including HIV infection (45%), solid organ transplant (26%), and cirrhosis (22%). Pulmonary cryptococcosis was diagnosed in 9 (28%) low-titer and 8 (25%) high-titer patients ($P = 1.00$). Disseminated cryptococcosis occurred in 7 (22%) low-titer and 15 (47%) high-titers cases ($P = 0.064$). Titers $\leq 1:10$ more frequently represented isolated antigenemia in HIV-positive than non-HIV, immunocompromised patients ($P < 0.001$). Follow-up testing in patients with $\leq 1:5$ titers ($n = 21$) showed persistently low titers in 6 of 12 instances and increased titers in 2 cases. Twenty-seven patients with low CrAg titers were treated with antifungal therapy and 22 (81%) responded well clinically. Low-serum CrAg titers ($\leq 1:10$) correlated with cryptococcal disease in a substantial proportion of non-HIV immunocompromised patients and should prompt careful clinical workup for cryptococcal infection.

KEYWORDS *Cryptococcus*, HIV, transplant

The pathogenic *Cryptococcus* species *C. neoformans* and *C. gattii* can cause disease after inhalation of spores, leading to a range of clinical manifestations, including asymptomatic antigenemia, isolated pulmonary disease, and disseminated and invasive infection of the skin, bone, lymph node, or central nervous system (CNS) (1). The vast majority of infected patients have clearly identified underlying immunocompromised conditions, such as HIV/AIDS or organ transplantation. The prevalence of cryptococcal antigenemia in HIV-infected patients (CD4⁺ T-cell count, $< 100/\mu\text{l}$) in low-income and middle-income countries is $\sim 6\%$ to 7% , with 1-year mortality in the range of 40% to 70% (2). In solid-organ transplant recipients, cryptococcal disease occurs in about 2.8% , with death rates as high as 49% among those with CNS disease (3–5). Rapid identification of cryptococcosis in these populations is paramount, as treatment decreases mortality (6).

Diagnostic testing for cryptococcal disease includes detection of the cryptococcal antigen (CrAg) in serum or CSF using latex agglutination, enzyme immunoassay, or

Citation Dizon E, Seo W, Butler-Wu SM, She RC. 2020. Clinical significance of low serum cryptococcal antigen titers by lateral flow assay in immunocompromised patients: a retrospective case-control study. J Clin Microbiol 58:e01648-19. <https://doi.org/10.1128/JCM.01648-19>.

Editor Geoffrey A. Land, Carter BloodCare and Baylor University Medical Center

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Received 1 October 2019

Returned for modification 3 November 2019

Accepted 8 November 2019

Accepted manuscript posted online 13 November 2019

Published 28 January 2020

lateral flow assay (LFA). LFA is a rapid test with increased analytical sensitivity and improved serotype coverage compared with latex agglutination (7, 8). Multiple published guidelines outline the effective therapy of clinically evident cryptococcal disease with high serum or CSF titers (3, 9, 10). However, in non-HIV immunocompromised patients, there is limited guidance on the treatment of asymptomatic cryptococcal antigenemia (3). Furthermore, there is ambiguity over the clinical significance of low CrAg titers by LFA given its increased analytical sensitivity compared with other methods (2, 11). We aimed to better understand the clinical significance of low-titer serum CrAg ($\leq 1:10$) by LFA in an immunocompromised patient population.

MATERIALS AND METHODS

Study design. All patients clinically tested for CrAg by LFA (IMMY, Norman, OK) in serum or CSF at Keck Medical Center and LAC+USC Medical Center (Los Angeles, CA) between 1 April 2014 and 31 July 2018 were retrospectively identified. The low-titer CrAg group included cases with first-time positive serum CrAg at titers of $\leq 1:10$ and excluded patients with history of cryptococcal disease. Patients with serum CrAg titers of $\geq 1:20$ and patients with negative CrAg results on initial diagnostic testing served as age-matched high titer-positive and negative-control groups, respectively. Age-matched controls were selected from the respective medical center location of the low-titer cases. Due to limited numbers of high-titer cases, criteria for age matching included cases ± 2 years of patient age.

Clinical chart review was performed by an investigator (E.D.) who was blind to the CrAg titer and other laboratory results of each case. Laboratory data were independently collected by investigator W.S. Data collected included patient demographics, immune status, comorbidities, reason for CrAg testing, radiologic findings, laboratory data at presentation, culture and histopathological results for *Cryptococcus* spp., clinical diagnosis, antifungal treatment, and response to therapy, including in-hospital mortality. The study protocol was reviewed and approved by our institutional review board.

Study definitions. Isolated cryptococcal antigenemia was defined as serum CrAg detection in the absence of symptoms attributable to cryptococcosis, with no culture recovery or histopathological documentation of *Cryptococcus* sp. from any site (12). A determination of pulmonary cryptococcal disease was based on the presence of symptoms, including cough and fever, dyspnea, or respiratory failure, and supporting chest radiograph or computed tomography findings of ground glass opacities or nodules in the setting of positive CrAg test once other disease etiologies had been ruled out (13). Disseminated infection was defined as cryptococcal disease involving two or more noncontiguous sites (3). This included meningitis, visceral disease, or skin and soft tissue manifestations as determined by the presence of symptoms or radiologic imaging along with a positive CSF CrAg assay, positive culture, or histopathological examination indicating cryptococcosis.

Statistical analysis. The sample size was not based on formal power estimation due to the known infrequency of patients with CrAg titers of $\leq 1:10$. For comparisons between groups, the two-tailed Fisher's exact test for categorical variables and Wilcoxon signed-rank test for continuous variables were used for analysis. Chi-square statistics were used to compare categorical data across the three study groups. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Patient characteristics. A total of 2,196 patients were tested by LFA for serum CrAg, of which 1,994 had negative results. Of the 202 CrAg-positive patients, 7 were excluded for having a history of cryptococcal disease from elsewhere, 163 (81%) had initial positive serum CrAg of $\geq 1:20$, and 32 (16%) had initial positive serum CrAg of $\leq 1:10$ (Fig. 1). Among these 32 patients with CrAg titers of $\leq 1:10$, 11 had a titer of 1:10, 17 were 1:5, and 4 were 1:2. Age-matched controls were identified for high-titer and negative-control groups ($n = 32$ each). All patients in the low- and high-titer groups had one or more immunocompromising conditions, including HIV/AIDS, cirrhosis, end-stage renal disease, diabetes mellitus, autoimmune disease, malignancy, and history of solid organ transplantation, including heart, liver, or kidney. In the negative-control group, 5 patients were immunocompetent and the remaining 27 were immunocompromised (Table 1). Chest radiography preceded obtainment of CrAg titer in all study patients except for 8 (8%), where evaluation prior to transplantation or starting chemotherapy necessitated imaging and CrAg testing simultaneously. Among HIV-positive patients with concurrent positive CrAg and peripheral CD4⁺ T-cell count results, CD4 count was $<100/\mu\text{l}$ in 10 of 13 (77%) with low-titer CrAg and 12 of 14 (86%) with high-titer CrAg.

CrAg titers in pulmonary disease. Respiratory symptoms at presentation (cough, shortness of breath, tachypnea, or respiratory failure) and chest radiograph findings (ground glass opacities or pulmonary nodules) were noted in similar proportions in

FIG 1 Study patient selection. Between 1 April 2014 and 31 July 2018, CrAg LFA results were reviewed for inclusion criteria to identify 32 patients who had serum CrAg titers $\leq 1:10$ on initial testing. Equal numbers of patients with high titer serum CrAg ($\geq 1:20$) and negative serum CrAg results were chosen as age-matched controls.

each group (Table 1). There were 35 cases in the low- and high-titer groups that also had pulmonary radiographic findings, of which 17 (49%) were diagnosed with pulmonary cryptococcosis. The frequency of pulmonary cryptococcosis was similar between low- and high-titer groups (9/32 [28%] versus 8/32 [25%] respectively, $P > 0.99$). The 10 low CrAg titer patients with positive chest radiographic findings that could not be

TABLE 1 Patient characteristics compiled for each study group^a

Characteristic	Values ^b by titer			P value
	$\leq 1:10$	$\geq 1:20$	Negative	
Mean age (yrs)	48.9	49.8	48.3	NS
Male	21 (66)	23 (72)	23 (72)	0.82
Immunosuppression type				
Cancer	3 (9)	3 (9)	7 (22)	0.24
Solid organ transplant	7 (22)	14 (44)	4 (13)	0.01 ^c
HIV/AIDS	13 (41)	15 (47)	15 (47)	0.84
Renal disease	4 (13)	11 (34)	4 (13)	0.04
Autoimmune disease	3 (9)	1 (3)	1 (3)	0.407
Cirrhosis	8 (25)	7 (22)	6 (19)	0.83
Diabetes mellitus	3 (9)	6 (19)	6 (19)	0.49
Clinical presentation				
Respiratory symptoms	13 (41)	8 (25)	11 (34)	0.41
Fever/chills	6 (19)	12 (38)	6 (19)	0.14
Confusion, headache, or seizures	12 (38)	17 (53)	10 (31)	0.19
Asymptomatic or planned admission ^d	3 (9)	2 (6)	4 (13)	0.69
Abdominal pain or diarrhea	6 (19)	9 (28)	4 (13)	0.29
AIDS	3 (9)	0 (0)	0 (0)	NA
Radiographic findings				
Nodule or ground glass opacities	19 (60)	16 (50)	14 (44)	0.45
No disease	11 (34)	16 (50)	16 (50)	0.39
Not performed	2 (6)	0 (0)	2 (6)	NA
Peripheral WBC count mean ($\pm$ SD), range ($\times 10^6$ /liter)	6.0 (± 3.8), 2.0–21.4	7.2 (± 5.9), 1.5–21.7	7.2 (± 4.0), 0.22–16.3	NS
In-hospital mortality	5 (16)	6 (19)	2 (6)	0.31

^a $n = 32$ each. $P > 0.05$ for each pairwise comparison. NA, not applicable; NS, not significant; WBC, white blood cell.

^bAll values are no. (%) except where indicated.

^c $P = 0.11$ for $\leq 1:10$ titer versus $\geq 1:20$ titer group and $P = 0.01$ between $\geq 1:20$ titer and negative titer group.

^dReasons included organ transplantation and cancer chemotherapy.

TABLE 2 Subanalysis of low-titer cases where serum CrAg titers were $\leq 1:5^a$

Characteristic	No. (%)
Immunosuppression type	
Cancer	2 (10)
Solid organ transplant	6 (29)
HIV/AIDS	10 (47)
Renal disease	3 (14)
Autoimmune disease	1 (5)
Cirrhosis	5 (24)
Diabetes mellitus	3 (14)
Radiographic Data	
Nodule or ground glass opacities	17 (81)
No disease	4 (19)
Not performed	0 (0)
Presumed site of cryptococcal infection	
Pulmonary	6 (29)
Disseminated	3 (14)
Isolated antigenemia	12 (57)
Culture or histopathology	
Positive for <i>Cryptococcus neoformans/gattii</i>	1 (5)
Negative for <i>Cryptococcus neoformans/gattii</i>	17 (81)
Neither performed	3 (14)
Treated for cryptococcosis	18 (86)
Clinical response if treated	15/18 (83)

^a*n* = 21.

attributed to *Cryptococcus* spp. either expired prior to additional workup, had lesions attributable to prior noncryptococcal granulomatous disease, or were diagnosed with multifocal bacterial pneumonia. There were 2 cases of histopathology-proven pulmonary cryptococcosis, 1 with 1:5 and another with 1:10 titer, where cultures (CSF, blood, and/or bronchoalveolar lavage) were negative. Among low-titer cases, pulmonary cryptococcosis was more likely to be diagnosed in non-HIV, immunocompromised patients (9 of 19, 47%) than in HIV-positive patients (0 of 13) ($P = 0.004$).

CrAg titers in disseminated cryptococcosis. Disseminated cryptococcal disease occurred in 15 (47%) cases in the high-titer group compared with 7 (22%) in the low-titer group, but this difference did not reach statistical significance ($P = 0.064$). More than one-third of patients in both high- and low-titer groups presented with confusion, headache, altered mental status, or seizures (17/32 [53%] and 12/32 [38%] respectively). Of the 7 cases of disseminated disease in the low-titer group, 6 were non-HIV immunocompromised patients and 1 had HIV infection. Four had titers of 1:10, 2 had $\leq 1:5$, and 1 patient had a 1:2 serum titer and was treated for disseminated cryptococcosis for disease involving the lungs and peritoneum.

CrAg titers in isolated antigenemia. Positive CrAg results were attributed to isolated antigenemia in 16 (50%) patients in the low-titer group compared with 9 (28%) patients in the high-titer group ($P = 0.12$). On subset analysis (Table 2), isolated antigenemia in patients with $\leq 1:5$ titers (12/21, 57%) was significantly more frequently observed compared with those with $\geq 1:20$ titers (9/32, 28%) ($P = 0.047$) but did not reach statistical significance compared with those with $\geq 1:10$ titers (13/43, 30%) ($P = 0.056$). Non-HIV immunocompromised patients were less likely to have isolated antigenemia than HIV-positive patients overall ($P < 0.001$), and this difference remained on separate subset analysis of titers $\leq 1:10$ (4/19 [21%] versus 12/13 [92%], respectively, $P < 0.001$) and $\geq 1:20$ (1/17 [6%] versus 8/15 [53%] respectively, $P = 0.005$).

Laboratory-confirmed cryptococcal disease. Significantly fewer patients in the low-titer group had culture-confirmed ($n = 3$) or histopathology-confirmed ($n = 2$) cryptococcosis (5/32, 16%) compared with patients in the high-titer group (13/32 [41%], $P = 0.050$). Of all culture-confirmed cases, only 1 was identified as *C. gattii* (serum CrAg

titer 1:80) and the remaining were *C. neoformans* ($n = 15$). In the low-titer group, all 5 laboratory-confirmed cases (3 respiratory and 2 CSF specimens) were in non-HIV-positive patients and included 2 with serum titers of 1:5. The frequency of culture or histopathology confirmation for any CrAg-positive case was significantly higher in disseminated disease than pulmonary cryptococcosis (15/22 [68%] versus 3/17 [18%] respectively, $P = 0.003$) and included positive cultures from CSF ($n = 12$), blood ($n = 9$), and peritoneal fluid ($n = 1$).

Treatment and follow-up. Antifungal therapy was initiated to treat cryptococcosis at the time of positive CrAg results on all but 5 patients with low CrAg titers (27/32, 84%). Of these 5 patients, 4 were diagnosed with isolated antigenemia and 1 was diagnosed with pulmonary cryptococcosis; 3 were HIV-positive and treated with fluconazole at the time of visit for candida esophagitis ($n = 2$) or at a subsequent visit after serum CrAg titer had increased to 1:80 ($n = 1$). No sequelae from cryptococcal disease were reported for these patients at 6 months after presentation. The fourth patient, who was preliver transplant, was treated with fluconazole 10 months later with subsequent CrAg seronegativity but persistence of pulmonary nodules and hilar lymphadenopathy. The fifth patient had interstitial lung disease believed to be secondary to hypersensitivity to pet birds and had a persistent cough despite treatment with immunosuppressives. She did not receive antifungals and was lost to follow up 3 months after presentation. No inpatient infectious disease consultations were placed for these patients.

Of the 27 patients who received antifungal therapy for cryptococcosis, 22 (81%) showed clinical improvement. All patients with high CrAg titers were treated with antifungal therapy for cryptococcosis, of whom 22 responded well clinically. In the low-titer group, follow-up CrAg LFA testing was ordered in 18 of 32 (56%) cases which occurred between 2 weeks and 10 months after initial testing. In all but one case, the repeat titer was obtained after starting antifungal therapy. Those who did not receive repeat testing either died or were lost to follow-up. Seven of the 18 patients with follow-up testing after a 1:5 titer showed persistently low titers ($\leq 1:5$), of which 3 patients had HIV/AIDS, 3 patients were solid-organ transplant recipients, and 1 patient suffered cirrhosis.

DISCUSSION

The aim of this retrospective study was to assess the clinical relevance of low serum CrAg titer ($\leq 1:10$) by LFA when evaluating for cryptococcal disease in immunocompromised patients. Because individuals with impaired cell immunity are at greatest risk of infection (4), detection of CrAg in the immunocompromised patient prompts clinical concern for cryptococcal disease. However, given the high analytical sensitivity of LFA and concern that low-titer LFA results potentially represent asymptomatic antigenemia (14, 15), the clinical significance of low-titer LFA results in immunocompromised patients remains an open question.

Our study included HIV and non-HIV immunocompromised patients, and we found that half of patients with low-titer serum CrAg ($\leq 1:10$) had documented clinical disease while half had isolated antigenemia. Pulmonary cryptococcosis occurred at similar rates in low- and high-titer groups. Disseminated disease, however, was more common at LFA titers of $\geq 1:20$ than $\leq 1:5$. Serum titers have been correlated with disease burden at time of presentation (16, 17), and we also found that culture or histopathological confirmation of cryptococcal disease occurred significantly more frequently in our high-titer than low-titer cases. Conversely, it is notable that disseminated disease was still diagnosed in patients with serum titers as low as 1:2 or 1:5. Although not a focus of our study, the false-negative rate of serum CrAg testing in cases of culture or histopathologically proven pulmonary cryptococcosis has ranged from 20% in one study of solid-organ transplant patients which used latex agglutination (16) to 34% in a study of predominantly immunocompetent patients which used LFA (18). These findings serve to emphasize that careful clinical evaluation and incorporation of radiologic

and laboratory data are necessary to rule out isolated antigenemia and symptoms attributable to other causes.

A clinical interpretation of isolated antigenemia in this study was not limited to patients with the $\leq 1:5$ titers but was noted in roughly one-third of immunocompromised patients with CrAg titers of $\geq 1:20$. In our study population, HIV-positive patients with low CrAg titers were more likely to have isolated antigenemia and less likely to have pulmonary cryptococcosis than non-HIV immunocompromised patients. This observation is likely due to the differing circumstances under which serum CrAg is ordered in these patients at our institution. HIV-positive patients with low CD4 count were frequently screened for cryptococcosis by serum CrAg, as evident by the high frequency of CD4 counts of $<100/\mu\text{l}$. Among non-HIV immunocompromised patients, serum CrAg was commonly ordered to work up chest imaging findings, thus increasing the likelihood of a positive CrAg result being attributed to pulmonary disease.

Among AIDS patients, asymptomatic antigenemia has been studied extensively and found to be associated with worse outcomes even in the absence of overt symptoms (19–21). In non-HIV, immunocompromised patient populations, it is not yet established if asymptomatic antigenemia is prognostic of subsequent disease development. The incidence of asymptomatic antigenemia outside the HIV-positive population appears to be low but is not well studied. Generally, the analytical specificity of the LFA CrAg test is excellent, with a pooled estimated specificity of 98.1% (22). One study observed no CrAg-positive cases by latex agglutination after screening 294 hospitalized patients with cirrhosis (23), but few other studies have assessed the seropositive rate in other immunocompromised groups. Of concern, Dubbels et al. found that CrAg results by LFA were falsely positive in 34% of 38 patients from a mixed immunocompromised and immunocompetent population (15). Most of the false-positive cases occurred at a serum titer of 1:2 and less than half were immunocompromised patients. Our study expanded the focus on immunocompromised patients and only identified four cases with an initial serum titer of 1:2. We found that low-positive serum CrAg even at $\leq 1:5$ titers was frequently clinically significant (Table 2).

The clinical significance of pulmonary findings in immunocompromised patients may be difficult to ascertain. A large proportion of the patients tested for serum CrAg in our study presented with respiratory symptoms and demonstrated chest radiographic findings, prompting testing. Pulmonary cryptococcosis is challenging to diagnose given the suboptimal sensitivity of respiratory cultures, nonspecificity of radiologic findings, and uncertainty of low-titer serum CrAg results (15, 18, 24, 25). We found that regardless of culture confirmation or clinical signs of disease, the vast majority of patients in the low-titer group were given antifungal therapy for treatment of cryptococcosis. In the absence of clear therapeutic guidelines for non-HIV patients, providers may extrapolate from HIV guidelines that suggest screening for asymptomatic cryptococcal antigenemia in advanced AIDS and treating with antifungal therapy as a cost-effective approach to prevent cryptococcal meningitis (14, 26, 27). Based on our clinical interactions with providers, concern over missing the diagnosis or starting treatment too late drives conservative decision-making in a severely immunocompromised patient.

Only 32 patients satisfied inclusion criteria for low-titer LFA in this retrospective investigation, but this is the largest study on low-titer CrAg in immunocompromised patients to date and included patients at two academic medical centers over a 4-year period. While we included age-matched controls, the cases were not further matched for sex or immunosuppressive states. A limited number of data elements were not available in some patient records as this was a retrospective analysis.

In conclusion, we report that low serum CrAg titer ($\leq 1:10$) by LFA in an immunocompromised population was frequently associated with documented clinical disease, particularly in non-HIV patients. The clinical practice at our institution was to treat immunocompromised patients with low serum titers despite low rates

of culture confirmation, with good clinical outcomes documented in the majority of patients. Careful clinical workup in immunocompromised patients should, therefore, be pursued to ensure exclusion of alternative diagnosis and avoidance of adverse effects associated with unnecessary antimicrobial usage.

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